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Two-Stage Hydrothermal Processing of Wheat (*Triticum aestivum*) Bran for the Production of Feruloylated Arabinoxylooligosaccharides

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Two-stage hydrothermal processing was employed to obtain feruloylated arabinoxylooligosaccharides (AXOS) from wheat bran. First, wheat bran in water (10% w/w solids) was heated to 130 °C, releasing 36.3% of total solids, 70.3% of starch, and 6.06% of pentose sugars. Wheat bran was then heated to 170−220 °C. Heating to 200 and 210 °C released the most AXOS (70% of the insoluble arabinoxylan) and esterified ferulate (30% of the initial ferulic acid). Treatment of 200 °C retained a higher proportion of high molecular weight (>1,338) compounds than 210 °C and was the preferred treatment temperature because autohydrolysate liquors contained lower concentrations of many contaminants. Purification of this autohydrolysate liquor with ethyl acetate extraction, vacuum concentration, and ion exchange resulted in a product containing 32.0% AXOS and 4.77% esterified ferulate, accompanied by 36.0% other oligosaccharides and free sugars, with an antioxidant activity of 29.7 μmol Trolox equivalents/g dry matter.

KEYWORDS: Antioxidant; autohydrolysis; ferulic acid; prebiotic; microwave; xylooligosaccharides

INTRODUCTION

Commercial wheat bran makes up about 11% of the grain and comprises the nuclear epidermis, seed coat, pericarp, aleurone layer, and adherent starchy endosperm (*1*). It consists of ash (6.1−6.5%), lipid (5.9−6.8%), protein (15−20%), starch (11−23%), dietary fiber (43−53%), and other minor constituents (*1*, *2*). The dietary fiber fraction is mostly insoluble, comprising lignin (5−20%), cellulose (16−30%), arabinoxylan (pentosan) (38−55%), and other nonstarch polysaccharides (*1*, *2*).

In the Unites States, about 5.7×10^9 kg of wheat bran were produced in 2008 (*3*), most of which was used for animal feed (*4*). Conversion of this byproduct of wheat flour milling to products of higher commercial value would be extremely valuable to the wheat industry.

To this end, much research has been devoted to harvesting the arabinoxylan fraction as soluble, feruloylated arabinoxylooligosaccharides (AXOS) (*5*−*7*). Feruloylated AXOS are principally oligosaccharides of β(1→4)-linked xylopyranosyl units of the degree of polymerization 2−9 or more with arabinofuranosyl side groups, some of which are substituted with ferulic acid (*5*−*7*). A growing body of research suggests that these oligosaccharides confer numerous health benefits, including prebiotic properties (*8*, *9*) and antioxidant activity (*6*, *10*).

AXOS from wheat bran have most commonly been produced through xylanase treatment (*5*−*7*). Depending on enzyme source and hydrolysis conditions, xylanases are effective at releasing about 30−40% of insoluble arabinoxylan (*7*, *11*). A potential alternative to enzymatic hydrolysis may be autohydrolysis (*12*). This process uses high pressure hydrothermal processing to release the insoluble arabinoxylan. This process does not require expensive enzymes or other chemicals for production, but purification of autohydrolysate liquors and equipment can be costly (*12*).

To improve the purity of autohydrolysate liquors and enhance their susceptibility to refining treatments, two-stage processes are sometimes used: the first stage intended to remove much of the contaminants such as low molecular weight phenolics and soluble inorganic compounds, and the second stage to liberate the insoluble arabinoxylan (*12*, *13*). The purpose of this research was to use two-stage hydrothermal processing to obtain feruloylated AXOS from wheat bran without the use of enzymes.

MATERIALS AND METHODS

Wheat Bran. Bran from hard red wheat was obtained from New-Organics (Greenacres, FL, USA) and ground to <1 mm by passing through a pin mill (160Z, Alpine, Augsburg, Germany) three times at 14,000 rpm with 5 min between each milling step.

Hydrothermal Pretreatment. Throughout this article, hydrothermal pretreatment refers to the first stage of hydrothermal processing, which was intended to remove unwanted solids and starch from wheat bran, while leaving the insoluble arabinoxylan largely intact.

Five grams (dry matter, dm) of wheat bran were weighed into a 100 mL perfluoroalkoxy, Teflon reactor vessel, and water was added to make a total of 50 g. The vessels were then sealed with lids that contained temperature probes that reached down into the slurry. Under constant stirring (300 rpm), the mixture was then heated in a special microwave (Ethos 1600, Milestone Inc., Monroe, CT, USA) with sufficient microwave energy

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to raise the temperature from 20 °C (room temperature) to final temperatures of 110 to 160 °C at 10 °C/min. This temperature program was controlled and monitored by EasyWave software (version 3.5.4.1, Milestone Inc., Monroe, CT, USA). The slurries were then immediately cooled to < 80 °C according to the manufacturer's instructions. Following treatment, the slurries were centrifuged at 1500*g* for 10 min and the supernatants retained. The insoluble materials were washed three times with water, and the water washings were combined with the original supernatants to make the final extracts, which were assayed for total solids, neutral sugars, and pH. The insoluble fractions were freeze-dried and assayed for total solids, neutral sugars, and starch.

The optimum temperature for hydrothermal pretreatment was selected as the treatment temperature that minimized the release of pentosans (sum of arabinose and xylose as they occur in a polysaccharide), while maximizing the release of starch, and total solids.

Autohydrolysis. The second stage of hydrothermal processing was referred to as autohydrolysis, wherein wheat bran that had been hydrothermally pretreated under selected conditions was subject to a second, more severe, hydrothermal process designed to liberate insoluble arabinoxylan.

Wheat bran was subjected to the hydrothermal pretreatment as determined above. The remaining insoluble portion of the wheat bran was quantitatively transferred back into the reactor vessel and made to a total of 50 g with water. The slurry was again heated in the microwave oven, except temperatures ranged from 170 to 220 °C. Liquors were analyzed for total neutral sugars, soluble oligosaccharides, free neutral sugars, free and esterified ferulic acid, furfural, starch, and molecular weight profiles.

Purification. The treatment that resulted in the highest release of AXOS was repeated 10 times and pooled to obtain a large volume of autohydrolysate liquor. The liquor was then extracted with ethyl acetate at a liquor/solvent volume ratio of 1:3 (v/v) (*13*), followed by vacuum concentration to about 20% of the original volume to concentrate the solids and remove volatile compounds. The concentrated liquor was then treated with Amberlite IRA 400 ion-exchange resin at a liquor/resin ratio of 20:1 (v/w) with mild agitation overnight (*14*). The ion-exchange resin was then removed by vacuum-assisted filtration (Whatman GF/A) and freeze-dried. The purified solids were assayed for total neutral sugars, soluble oligosaccharides, free neutral sugars, free and esterified ferulic acid, and antioxidant activity.

Analytical Methods. Total solids of wheat bran raw material were determined according to approved method 44-15.02 (*15*). Total solids of autohydrolysate liquors and insoluble materials were determined by calculating the loss in weight upon freeze-drying a portion (about 15 g) of the extract or the entire insoluble material.

Total neutral sugar composition of solid samples (wheat bran starting material and insoluble solids after autohydrolytic treatment) was determined on 50 mg of sample following previously published steps for hydrolysis, derivitization, and gas−liquid chromatographic analysis (*16*). In liquid samples, total neutral sugars, oligosaccharides, and free neutral sugars were determined after Rose and Inglett (*17*). Using this method, oligosaccharides were defined as oligomers of degree of polymerization (DP) two or higher. Although this is different from the traditional definition of oligosaccharides (*18*), it is an accepted practice when referring to autohydrolysate liquors, even though some of the longer chain oligosaccharides may more appropriately be called polysaccharides (*19*, *20*). Nonstarch glucan was determined by subtracting the total glucan content from that contributed by starch.

In solid samples (wheat bran starting material and the insoluble solids after hydrothermal treatment), starch was determined using an enzymatic method (AA/AMG method, Megazyme, Bray, Ireland). In soluble samples (autohydrolysate liquors), this method was modified. Specifically, liquor containing 5 mg of equivalent carbohydrate (sum of total neutral sugars) was weighed into each of two tubes and diluted to 1 mL with water. One-half milliliter of 3-(*N*-morpholino)propanesulfonic acid buffer (0.15 M containing, pH 7.0, containing 0.015 M calcium chloride) was added to both tubes, and 20 U of thermostable α-amylase (Megazyme, Bray, Ireland) was added to one of the tubes. Capped tubes were placed in boiling water for 10 min and cooled. One-half milliliter of sodium acetate buffer (0.4 M, pH 4.5) was then added to both tubes, and 2 U of amyloglucosidase (Megazyme, Bray, Ireland) was added to the tube that had originally received α-amylase. Capped tubes were incubated at 50 °C for 30 min, followed by boiling for 10 min to inactivate enzymes. After centrifugation (3000*g*, 10 min), glucose was measured in all tubes using an enzymatic method (GOPOD format, Megazyme, Bray, Ireland). Starch was determined by subtracting the glucose content of the tube without enzyme treatment from that of the tube that was treated with amylolytic enzymes.

Free and esterified ferulic acid and furfural were determined according to Rose and Inglett (*17*). To determine the percent conversion of pentosans to furfural, the equation of Carvalheiro et al. (*21*) was used.

The molecular weight profiles of autohydrolysate liquors were determined after the removal of starch using size-exclusion chromatography (SEC). To remove starch, liquor containing 10 mg of equivalent carbohydrate (sum of total neutral sugars) was weighed into a tube, and 0.5 mL of 50 mM hydrochloric acid was added to bring the pH to between 1 and 2. Sixty-two and one-half microliters of pepsin (P7000, Sigma-Aldrich, St. Louis, MO, USA, 4 mg/mL in 50 mM hydrochloric acid) was then added, and the tube was incubated with horizontal shaking (150 rpm) at 37 °C for 30 min. Two milliliters of 0.1 M acetate buffer (pH 5.2 containing 4 mM calcium chloride) and 62.5 μL of an enzyme cocktail containing pancreatin, amyloglucosidase, and invertase, prepared according to Englyst et al. (*22*), were then added and the tubes incubated with horizontal shaking (150 rpm) at 37 °C for 2 h. Following digestion, the tubes were boiled to inactivate enzymes, made to a final volume of 5 mL, centrifuged (3000*g*, 10 min), and filtered through a 0.45 μm membrane. The digested solution (100 μL) was then injected onto a SEC (150-CV plus, Waters, Milford, MA, USA) equipped with a Shodex SB-803 HQ column preceded by a Shodex SB-G column (Showa Denko K.K., Tokyo, Japan). The column temperature was maintained at 35 °C, and the mobile phase was 0.02% sodium azide at 0.5 mL/min. Detection was carried out using a refractive index detector, and data were collected using Millennium 2010 software (Waters, Milford, MA). Molecular weights were estimated using glucan standards of molecular weights 4.80×10^4, 2.37×10^4, 1.22×10^4, 5.80×10^3 (Polymer Laboratories, Amherst, MA, USA), 8.29×10^2, 5.04×10^2, and 1.80×10^2 (284033, 851493, and 158968, respectively, Sigma-Aldrich, St. Louis, MO, USA).

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazil (DPPH) method as described by Glucin (*23*) with minor modifications. Briefly, the purified sample was dissolved in water and diluted to about 0.5 μg dm/mL. Then, 1.8 mL of water and 0.1 mL of ethanol was added, followed by 1 mL of 0.1 M DPPH solution in ethanol. After 30 min in the dark at room temperature, the absorbance was recorded at 517 nm. Results were expressed as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents.

Data Analysis. Data were analyzed using SAS software (version 8, SAS Institute, Cary, NC, USA) with a general linear model analysis of variance. Significant differences among treatment combinations were determined using Fisher's least significant difference adjustment.

RESULTS AND DISCUSSION

Wheat Bran. The wheat bran used in this study contained (% dm) 24.4 ± 0.2 starch, 18.4 ± 0.0 total pentosans (sum of arabinose and xylose as they occur in a polysaccharide), 6.67 ± 0.63 nonstarch glucan (total glucan less that contributed by starch), 2.40 ± 0.05 galactan, and 0.54 ± 0.03 ferulic acid. The starch content was a little higher, and the nonstarch glucan content was a little lower than that typically found in wheat bran (11−23% starch and 8−16% nonstarch glucan) (*1*, *2*), which was likely due to the noticeable endosperm contamination in the wheat bran from this manufacturer. The remainder of the wheat bran was assumed to be protein (15−20%), lipid (5.9−6.8%), lignin (5−20%), ash (6.1−6.5%), and other compounds typically associated with wheat bran (*1*, *2*).

Hydrothermal Pretreatment. Wheat bran was first treated under moderate hydrothermal conditions to prepare the wheat bran for autohydrolytic release of feruloylated AXOS. For this stage of processing it was desirable to maximize the release of total solids, maximize starch removal, and minimize the release of pentosans. Maximizing total solids and starch removal would ease purification and result in a final product with a higher percentage of feruloylated AXOS (*12*, *13*). Minimizing the release of pentosans would allow for higher product yields since the arabinoxylan released during this stage of processing was discarded.

Although statistically significant, only small increases in total solids were experienced as processing temperature increased (**Table 1**). The removal of starch also increased with increasing temperature; however, no significant increase in starch removal was experienced beyond a final temperature of 130 °C. At processing temperatures of 110−130 °C, relatively small increases in the release of pentosans was observed (about 0.5% for each increment), whereas between 130 and 160 °C, larger increments were seen (> 1%). Wheat bran generally contains 0.63 to 1.52% soluble pentosans (*24*), which is about 3 to 7% of the total pentosan content. These pentosans may be present in the form of soluble arabinoxylan or arabinogalactan peptide (*25*, *26*). Treatment temperatures up to 130 °C clearly fall within the normal range for soluble pentosans. Therefore, the loss of pentosans during this step probably represented only the soluble portion of the total pentosan content; the insoluble arabinoxylan was likely unaffected. This is substantiated by comparing the pH of extracts: final treatment temperatures of 110−130 °C resulted in no significant change in pH, whereas at higher temperatures, the pH began to decrease. This suggests that autohydrolysis was not induced at temperatures up to 130 °C.

Taking these results into account, a final temperature of 130 °C was selected as the optimum pretreatment temperature as it did not induce autohydrolysis and resulted in among the lowest release of pentosans and highest release of starch, accompanied by a moderate release of solids.

Autohydrolysis. For the second stage of hydrothermal processing, which was intended to release feruloylated AXOS by autohydrolysis, wheat bran was subjected to the selected pretreatment temperature of 130 °C, washed, and then subjected to higher temperature processing. The pH of liquors following the second hydrothermal treatment decreased significantly as temperature increased (**Table 2**), suggesting the initiation of autohydrolysis (*12*). Concomitantly, solids liberated from wheat bran increased with increasing treatment temperature.

Figure 1 shows the fate of the insoluble arabinoxylan fraction of wheat bran after pretreatment. The optimum final temperatures for autohydrolytic release of AXOS from wheat bran were 200 and 210 °C. Under these conditions, about 70% of the insoluble arabinoxylan of wheat bran was converted to soluble AXOS. Similar percentages of insoluble arabinoxylan have been liberated from other agricultural products using autohydrolysis (*20*, *27*). This is substantially higher than the percentage of AXOS reportedly released by xylanases (*7*, *11*).

Despite similar percent conversion of insoluble arabinoxylan to AXOS at treatment temperatures of 200 and 210 °C, the structures of the AXOS appeared to be quite different. Because arabinoxylans consist, basically, of a xylose backbone with arabinose side chains (*25*), the arabinose to xylose A/X ratio is an estimate of the degree of branching. At 200 and 210 °C, the arabinose to xylose ratios of released AXOS were 0.39 ± 0.00 and 0.26 ± 0.00 (p for difference < 0.001). Therefore, AXOS released at 200 °C were more branched than those at 210 °C. The molecular weight distribution of released AXOS was also studied using SEC. Because SEC profiles revealed broad ranges of molecular weights, peaks were divided into low, medium, and high molecular weights. Low molecular weight corresponded to < 414 [degree of polymerization (DP) < 3 for arabinoxylans], while medium and high molecular weight referred to 414−1,338 (DP 3−10) and > 1,338 (DP > 10), respectively. These divisions were chosen because saccharides with DP of 3−10 usually constitute oligosaccharides, while DP < 3 or > 10 comprise

Table 1. pH of Extracts and Total Solids, Starch, and Pentosans Released from Wheat Bran upon Hydrothermal Treatment to Selected Temperatures[a]

temperature (°C)	final pH	total solids % released[b]	starch % released[c]	pentosans % released
110	6.34 (0.12) a	32.6 (0.5) e	59.5 (3.1) b	5.17 (0.04) e
120	6.31 (0.05) a	34.7 (0.8) d	64.6 (1.8) b	5.55 (0.04) de
130	6.24 (0.04) ab	36.3 (0.4) cd	70.3 (1.3) a	6.06 (0.12) d
140	6.22 (0.02) ab	37.6 (0.0) bc	72.3 (1.3) a	7.36 (0.28) c
150	6.11 (0.00) b	39.4 (0.7) ab	74.7 (0.9) a	8.78 (0.03) b
160	6.12 (0.02) b	39.7 (0.3) a	74.3 (0.6) a	11.3 (0.5) a

[a] Values are reported as mean (standard deviation); like lower case letters within the column indicate no significant difference ($p > 0.05$); $n = 2$. [b] Reported as a percentage of the dry weight of the starting material. [c] Starch and pentosans are reported as a percentage of the amount in the starting material.

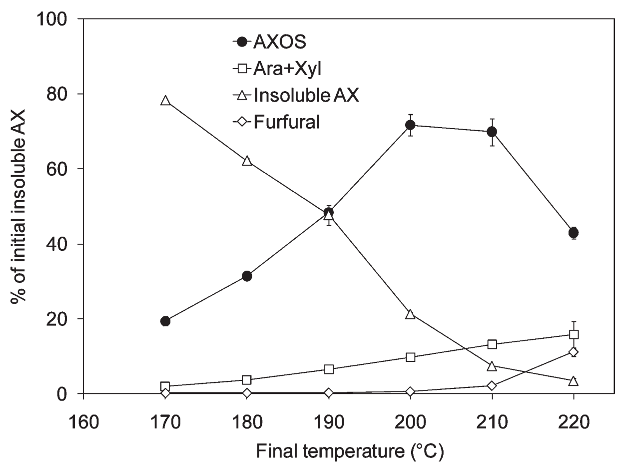

Figure 1. Fates of insoluble arabinoxylan (AX) during autohydrolytic treatment at selected temperatures. AXOS, arabinoxylooligosaccharides; Ara+Xyl, sum of free arabinose and xylose; error bars show standard deviation ($n = 2$); some error bars are too small to see.

Table 2. Final pH and Total Solids, Galactan, Starch, and Nonstarch (ns) Glucan Released during Autohydrolytic Treatment of Pretreated Wheat Bran[a]

temperature (°C)	final pH	total solids % released[b]	galactan % released[c]	starch % released	ns glucan % released
170	6.06 (0.35) a	20.0 (0.2) d	ND[d]	9.55 (0.70) a	35.8 (3.1) c
180	6.02 (0.04) ab	25.7 (3.4) d	ND	8.83 (1.32) a	26.6 (0.9) d
190	5.84 (0.15) ab	37.3 (4.5) c	31.0 (2.4) b	4.52 (0.13) bc	35.1 (2.7) cd
200	5.54 (0.06) b	53.8 (3.8) b	38.0 (3.9) ab	3.78 (0.43) c	41.9 (2.7) bc
210	5.00 (0.06) c	62.7 (0.3) a	46.2 (0.6) a	4.06 (0.41) bc	47.9 (4.4) ab
220	4.33 (0.31) d	61.3 (0.2) a	42.1 (6.3) a	5.71 (0.52) b	53.0 (5.3) a

[a] Values are reported as mean (standard deviation); like lower case letters within the column indicate no significant difference ($p > 0.05$); $n = 2$. [b] Reported as a percentage of the dry weight of the pretreated solids. [c] Starch, ns glucan, and galactan are reported as a percentage of the amount in the pretreated solids. [d] Not detected.

Table 3. Relative Molecular Weight Distributions of Destarched Autohydrolysate Liquors[a]

temperature (°C)	molecular weight <414	414−1,338	>1,338
170	43.2 (3.5) ab	26.3 (0.6) a	30.6 (2.9) c
180	40.4 (0.1) ab	25.4 (2.0) a	34.1 (2.0) bc
190	37.7 (1.8) bc	24.2 (2.6) a	38.1 (0.8) b
200	31.6 (5.4) c	26.0 (4.3) a	42.4 (1.1) a
210	43.5 (2.3) ab	20.2 (1.2) a	36.3 (1.1) b
220	44.8 (0.3) a	24.2 (1.0) a	31.0 (1.3) c

[a] Expressed as % of total area (standard deviation) obtained from size-exclusion chromatography profiles; like lower case letters within the column indicate no significant difference ($p > 0.05$); $n = 2$.

mono- and disaccharides and polysaccharides, respectively (*18*). Treatment at 200 °C resulted in the retention of a higher percentage of high molecular weight compounds compared to treatment at 210 °C, whereas the converse was true for low molecular weight compounds (**Table 3**). No significant differences were discovered in the relative percentages of medium molecular weight compounds (414−1,338) among the different treatment temperatures.

The differences between AXOS structures may have effects on physiological properties, should these products be used a food ingredients. Craeyveld et al. (*8*) tested the effects of the degree of branching and molecular weight of AXOS on gut health in rats. They found that the degree of branching had minimal effect on intestinal characteristics such as short chain fatty acid production and concentrations of bifidobacteria; however, low molecular weight AXOS (average DP of 5) were bifidogenic and increased acetate and butyrate production in the cecum and colon, while high molecular weight AXOS (average DP of 61) decreased branched chain fatty acid production.

Galactan also appeared in autohydrolysate liquors (**Table 2**). Galactose in wheat bran occurs in arabinogalactan peptide and as a minor component of arabinoxylan (*25*, *26*). Arabinogalactan peptide is soluble (*26*) and thus was likely removed during the pretreatment. This was supported by the observation that galactan was not detected in autohydrolysate liquors at the lower final treatment temperatures (170 and 180 °C). When galactan was present in the autohydrolysate liquor, it represented 7−9% of the measured amount of AXOS. This is in the range reported for galactan content of insoluble arabinoxylan in wheat bran (*25*). Therefore, the galactan in the autohydrolysate liquors was most likely a component of released AXOS.

In addition to small amounts of galactan, another component of insoluble arabinoxylan in wheat bran is ferulic acid, which is attached to the polymer via an ester linkage to arabinofuranosyl units (*5*). Oligosaccharides released from agricultural products largely retain this feruloyl moiety (*12*) and are, therefore, often referred to as feruloylated oligosaccharides (*6*, *10*). This substituent may impart significant antioxidant activity to AXOS from wheat bran (*6*, *10*). Ferulic acid that was attached to soluble AXOS was qualitified my measuring the ferulic acid that was released from autohydrolysate liquors upon alkaline saponification. Not surprisingly, the highest concentrations of esterified ferulate in autohydrolysate liquors (**Figure 2**) occurred at the same optimum temperatures for AXOS release. This was accompanied by a small release of free ferulic acid.

Not all of the insoluble arabinoxylan degraded to AXOS; some appeared in autohydrolysate liquors as free sugars or degraded further to furfural (**Figure 1**). Arabinose and xylose were the only free sugars detected in autohydrolysate liquors. Arabinose always occurred at higher concentrations than xylose (not shown),

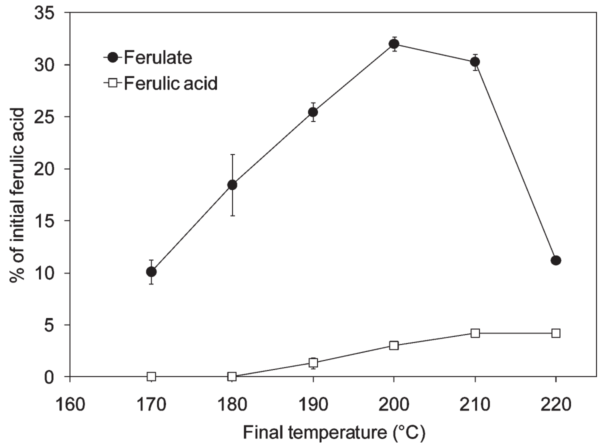

Figure 2. Release of ferulic acid as esterified ferulate or free ferulic acid during autohydrolytic treatment at selected temperatures. Error bars show standard deviation ($n = 2$); some error bars are too small to see.

indicating that arabinoxylan was partially debranched during autohydrolysis. At the optimum temperatures for AXOS release, 200 and 210 °C, similar percentages of free sugars appeared in autohydrolysate liquors. However, at 210 °C, more insoluble arabinoxylan was degraded to furfural than at 200 °C. Therefore, 200 °C may be more desirable than 210 °C due to less pentose sugar degradation. Furthermore, at the highest treatment temperatures (210 and 220 °C), the insoluble arabinoxylan likely degraded to compounds in addition to furfural, such as hydroxymethylfurfural and volatile compounds (*13*), because the sum of products shown do not add to 100%.

The presence of starch and nonstarch glucan in autohydrolysate liquors was undesirable because these are not components of AXOS. Starch remaining in autohydrolysate liquors represented 3.78 to 9.55% of the residual starch content of the pretreated wheat bran and did not change as a function of treatment temperature (**Table 2**). The treatment temperatures that resulted in the highest release of AXOS resulted in among the lowest concentrations of starch. As mentioned, the wheat bran used in this study contained an unusually high concentration of starch; therefore, using wheat bran with lower starch content initially may result in less starch in the final autohydrolysate liquors. Surprisingly, autohydrolysate liquors contained substantial amounts of nonstarch glucan; at the maximum final temperature (220 °C), 53.0% of the nonstarch glucan content of the pretreated wheat bran was released. This nonstarch glucan may have originated from cellulose or insoluble, mixed-linkage β-glucan (the small amount of soluble β-glucan was likely removed during pretreatment). Usually cellulose is largely unaffected by autohydrolytic treatments (*12*); however, these data suggest that the cellulose in wheat bran may be more affected by high temperature processing than that of other agricultural products. Alternatively the variety of wheat bran used in this study may have contained an unusually high content of mixed-linkage β-glucan.

Purification. Despite the hydrothermal pretreatment, autohydrolysate liquors still contained a number of contaminants. It was desirable to remove these contaminants if these AXOS preparations were desired for food use. Autohydrolysate liquors corresponding to pretreatment to 130 °C followed by autohydrolysis to 200 °C, which was the optimum treatment for releasing feruloylated AXOS with the highest molecular weight and lowest percentage of contaminants, were purified using ethyl acetate extraction, vacuum concentration, and treatment with anion exchange resin. These steps were intended to retain the saccharide components (i.e., sugars and sugar substituents) while removing other compounds (e.g., free phenolic compounds, volatile compounds, peptides, etc.). These procedures resulted in a product that contained (% dm) 32.0 ± 0.0 AXOS, 2.23 ± 0.07 galactan, and 4.77 ± 0.15 esterified ferulate (39.0% AXOS plus substituents). This was accompanied by 5.01 ± 0.11 free monosaccharides and 28.8 ± 0.3 glucan for a total of 72.8% dm. This is similar to purities reported previously (*28*). The remainder of the dm was likely made up of acid-soluble lignin and melanoidins (*28*). Other methods of refining, such as membrane processing (*12*, *13*), may be valuable for increasing the percentage of AXOS by removing free sugars and starch.

The material following autohydrolysate purification contained an antioxidant activity equivalent to 29.7 ± 0.9 μmol of Trolox/g dm. Antioxidant activities of water and alcohol-soluble extracts from other cereal grains, including oats, rice, wheat, millet, soybean, and distillers' dried grains are generally < 10 μmol of Trolox/g dm and more often < 5 μmol of Trolox/g dm (*29*, *30*). Therefore, the feruloylated AXOS in this study possess soluble antioxidant activities that may be as much as 3−6 times higher than extracts from untreated cereal products.

In conclusion, a two-stage hydrothermal process may be used to liberate insoluble arabinoxylan as feruloylated AXOS. The optimum treatments of wheat bran to release these compounds were 130 °C followed by 200 °C. These treatments resulted in about 70% of insoluble arabinoxylan release, accompanied by more than 30% of ferulic acid released as esterified ferulate. The liberated feruloylated AXOS contained a wide range of molecular weights and possessed antioxidant activity, which may impart important health benefits. Purification of autohydrolysate liquors with ethyl acetate, vacuum concentration, and anion exchange resulted in a product that contained 39% AXOS plus substituents. Improved methods of purification, in particular those aimed at removing starch and monosaccharides, are necessary before autohydrilytic release of feruloylate AXOS from wheat bran will become practical for use as a food ingredient.

SAFETY

Because care must be taken during the high pressure processing necessary to induce autohydrolysis, the safety portions of the instruction manuals accompanying both the mini benchtop reactor (4560, Parr Instrument Co., Moline, IL, USA) and microwave (Ethos 1600, Milestone Inc., Monroe, CT, USA) were consulted and heeded. The chemicals used in this study required no special safety considerations beyond basic laboratory safety.

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